LATITUDINAL EFFECTS ON METABOLIC RATES IN THE FROG, *ACRIS CREPITANS:* SEASONAL COMPARISONS

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In his review of latitudinal effects on the physiological properties of animal populations, Vernberg (1962) points out that a variety of physiological differences occur among latitudinally separated populations of some species. The differences are of considerable interest both to the comparative physiologist and to the ecologist, since they provide insight into the relative roles of longer term genetic changes in populations *versus* shorter term physiological changes of organisms in their adaptation to varying climatic regimes. Differences in whole body oxygen consumption which are apparently genetically fixed have been reported for a variety of organisms. Where these differences have been reported, they are such as to suggest that latitudinal compensation in metabolism for temperature occurs. In general, northern populations have a higher rate of oxygen consumption than southern populations of the same or related species when determined over a wide range of temperatures (Bullock, 1955; Prosser, 1955; Vernberg, 1962; Tashian and Ray, 1957; Hutchison, Whitford and Kohl, 1968).

Several investigators have compared the metabolic rates of latitudinally separated populations of the same species of anuran. Tashian and Ray (1957) compared northern and southern subspecies of *Bufo boreas* and found no significant differences between them. Jameson, Taylor and Montjoy (1970) however, report a great deal of variability in metabolic rates among populations of the frog, *Hyla regilla.* Dunlap (1972) reports that for warm acclimated *Acris crepitans,* South Dakota frogs have higher metabolic rates at low temperatures than do Texas frogs.

When comparing latitudinally separate populations one would hope to distinguish between short-term, environmentally induced variations in metabolic patterns and those that are genetically determined. However, the occurrence of marked seasonal changes in the metabolic rates of intact frogs which has been reported for a number of species, *e.g., Rana fusca* (Krogh, 1904); *R. esculenta* (Stangenberg, 1955); *R. pipiens* (Fromm and Johnson, 1955); *R. temporaria* (Dolk and Postma, 1927); *Acris crepitans* (Dunlap, 1969); *Hyla regilla* (Jameson *et al.,* 1970), can complicate the interpretation of comparative studies. That these changes are not due to short-term thermal acclimation to seasonal differences in temperature is evidenced by the fact that recent investigators who have reported the seasonal changes have compared animals acclimated at the same temperatures (Stangenberg, 1955; Dunlap, 1969; Jameson *et al.,* 1970).

There is little organized information available on the latitudinal effects on seasonal shifts in metabolic rates. However, in many Amphibia, including *Acris crepitans,* southern populations have a longer breeding season and a shorter period of dormancy than more northern populations (Stebbins, 1951). If the seasonal metabolic changes are related to the periods of reproduction and dormancy, one could

expect that the seasonal metabolic cycles of latitudinally separated populations would be out of phase with each other. When thermally acclimated samples of latitudinally separated populations are compared at some point in time and differences found, one cannot determine whether the observed differences are due to differences in the genotypes of the populations or simply to the comparison of two non-homologous points in a seasonal metabolic cycle in the acclimatized (*sensu* Prosser, 1958) populations. Conversely, the seasonal differences could conceivably mask genetically controlled differences between the populations.

Consequently, in view of the paucity of information concerning latitudinal effects and latitudinal-seasonal interactions on the metabolic rates of anurans, a comparison was made of the seasonal changes in the metabolic rates of acclimated cricket frogs from two latitudinally widely separated populations. The results were then compared with information on seasonal activities and environmental and body temperatures to see if there were correlations between metabolic patterns and the environmental temperatures which the frogs normally encounter.

Materials and Methods

Samples of cricket frogs were collected near Vermillion, South Dakota (Latitude 42° 48′ N; elevation 1220 ft) and Austin, Texas (Latitude 30° 18′ N; elevation 615 ft). Vermillion lies approximately 870 miles north of Austin.

Frogs from South Dakota were collected at intervals between April and early November, 1967 (Fig. 1), one to two days prior to placing them in the acclimation chambers. Texas frogs were collected at intervals between October and December, 1968, and between early April and early July, 1969 (Fig. 2). The Texas frogs were shipped to Vermillion *via* air express and were placed in the acclimation chambers upon arrival.

Groups of frogs were acclimated in the dark for 5–7 days (Dunlap, 1969) at temperatures of 5, 15 or 25 ± 1° C. They were maintained in loosely covered glass jars and had access to free water but were not fed during the course of acclimation.

Oxygen consumption was measured at 15° C for individual frogs using a refrigerated Gilson differential microrespirometer equipped with 100 ml flasks. Each flask received 5 ml deionized water in the animal chamber and carbon dioxide was absorbed by 1.5 ml of 20% KOH placed in the side arm. The flasks were equilibrated for 30 min and readings were taken every 15 min for 2 hr. Stability was routinely monitored by the insertion of a blank specimen vessel.

Metabolic rates are given as $\mu l/g$ per hr STP of oxygen and are based on the average hourly uptake over the 2 hr period. These can best be considered as routine rates in the sense of Fry (1957) inasmuch as there was no control of spontaneous locomotor activity during the 2 hr period of determination (Dunlap, 1971).

Animals collected at a particular time from each locality were assigned to the three acclimation temperatures on a random basis with the restrictions that there should be approximately equal proportions of males and females and equal proportions of three arbitrarily assigned size classes within each acclimation group. I was, however, unable to control size variation between samples taken at different times of the year. Furthermore, South Dakota frogs averaged considerably larger

(1.27 g) than the Texas frogs (0.90 g). Consequently, comparisons between samples from different times of the year or between samples from the two localities were made by direct comparison of metabolism-body weight regression lines following a log-log transformation of the data. The statistical techniques used in the analyses are from Li (1957) and Ostle (1963).
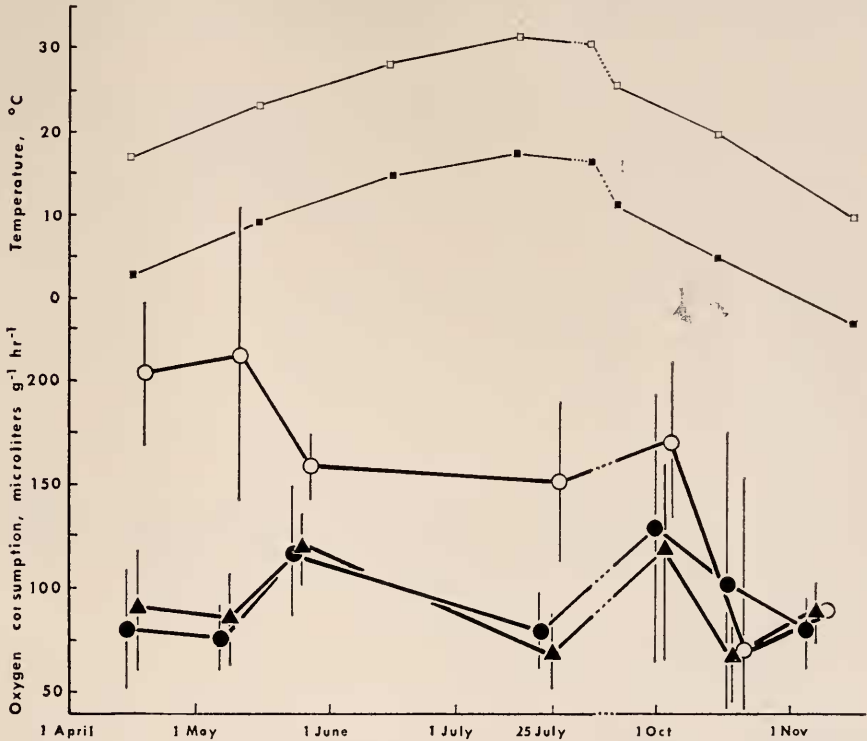

FIGURE 1. The three lower curves represent routine oxygen consumption in *Acris crepitans* from South Dakota at various times of the year determined at 15° C following acclimation of the frogs at 5, 15 and 25° C. Each point on the graph represents the predicted mean metabolic rate for frogs weighing 1.25 grams. The vertical lines represent the 95% confidence intervals for the predicted means. Symbols representing the acclimation temperatures are 5° C (filled circle); 15° C (filled triangle); 25° C (open circle). The upper pair of lines represent the mean maximum monthly temperatures (open square) and the mean minimum monthly temperatures (filled square) at Vermillion, S. D.

RESULTS

Mean metabolic rates of specimens of *Acris crepitans* acclimated at 5, 15 or 25° C and determined at 15° C at different times of the year are shown in Figures 1 and 2. With the exception of the mean metabolic rate of frogs acclimated at 25°C and determined on 6 November, which rate is based on only two animals, the means for the South Dakota frogs (Fig. 1) are based on samples of 5–7 animals. In all, these data are based on a total of 121 different frogs. The means

for the Texas speciments of *Acris* (Fig. 2) are based on 6–8 frogs except for the animals acclimated at 5° C and determined 8 April, 29 April, 4 October and 1 November which means are based on samples of 4–5 frogs. The Texas data are based on a total of 117 animals.

Since there was marked variation in the mean body weight of samples of frogs collected at different seasons, the mean metabolic rates shown in Figures 1 and 2 are corrected for body weight. The values for the South Dakota frogs shown in Figure 1 are calculated for frogs of 1.25 g body weight; for the Texas frogs the
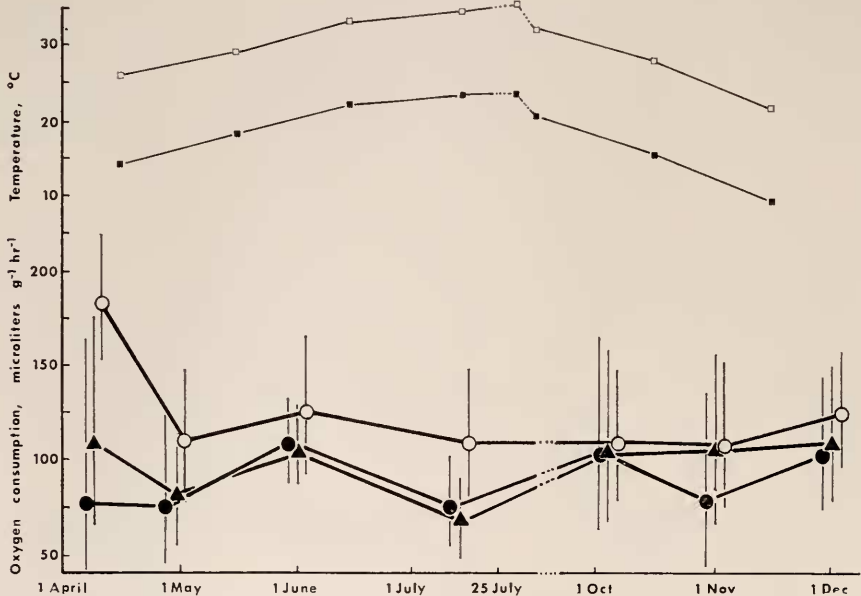

FIGURE 2. The three lower curves represent routine oxygen consumption in *Acris crepitans* from Texas at various times of the year determined at 15° C following acclimation of the frogs at 5, 15 and 25° C. Each point on the graph represents the predicted mean metabolic rate for frogs weighing 1.0 grams. The vertical lines represent the 95% confidence intervals for the predicated means. The upper pair of lines represent the mean maximum and mean minimum monthly temperatures at Austin, Texas. Symbols are as in Figure 1.

corrected means are for frogs of 1.00 g body weight. The 95% confidence intervals of the means are also shown in Figures 1 and 2. It should be borne in mind, however, that the variation in the width of the confidence intervals is due not only to the variation among variances of the samples but also to the variable sample size and to seasonal differences in the degree of deviation of mean body weights of the samples from the overall mean.

Acclimation effects

While there were marked differences in mean body size among samples collected on different dates, on any one date the samples were stratified with respect

to body weight and sex. Consequently, Student's t-values were calculated for all pairs of means on any one sample date. The results are shown in Table I. As may be seen, there is no statistically significant difference between the means of samples acclimated at 5 and 15° C ($P > 0.05$) on any date for either the South Dakota or Texas series.

No significant differences were found among the means of the three acclimation groups from October into December ($P > 0.05$) except for Texas frogs acclimated at 25 and 5° C in November ($P < 0.05$).

TABLE I

Student's t-values comparing the mean metabolic rates of samples of Acris crepitans *from South Dakota and Texas which had been acclimated at 5, 15 and 25° C and determined at 15° C on the dates indicated. The degrees of freedom for each calculated t-value is enclosed in parentheses.*

Locality	Temperatures compared	Date of determination						
		16 April	7 May	27 May	24 July	2 Oct	18 Oct	6 Nov
South Dakota	25–15° C	6.715** (10)	4.598** (10)	7.140** (10)	4.840** (10)	1.938 (10)	1.527 (8)	0.426 (7)
	25–5° C	7.356** (10)	5.362** (10)	8.590** (10)	4.674** (10)	0.851 (11)	0.798 (9)	0.334 (7)
	15–5° C	0.691 (10)	1.926 (10)	1.509 (10)	0.342 (10)	1.204 (9)	2.850 (9)	1.248 (12)
		8 April	29 April	31 May	20 July	4 Oct	1 Nov	3 Dec
Texas	25–15° C	3.08* (9)	2.936* (10)	1.78 (14)	4.557** (10)	1.16 (11)	0.045 (11)	2.08 (10)
	25–5° C	3.24* (8)	3.860** (9)	1.11 (12)	3.420** (10)	0.65 (9)	2.73* (8)	1.68 (11)
	15–5° C	2.02 (7)	0.412 (9)	0.617 (12)	1.898 (10)	1.44 (8)	1.54 (9)	0.367 (11)

* $P < 0.05$.
** $P < 0.01$.

From April through July the South Dakota frogs acclimated at 25° C had metabolic rates which were consistently higher than and significantly different from ($P < 0.01$) either the 5 or 15° C acclimation groups. With the exception of the samples of 31 May ($P > 0.05$), the metabolic rates of Texas frogs acclimated at 25° C were higher than and significantly different from ($P < 0.05$) those acclimated at 5 and 15° C in April through July.

Seasonal effects

In an effort to determine whether there are significant seasonal variations among samples from one locality, the metabolic data from samples of frogs which had been acclimated at the same temperature were compared. Inasmuch as the mean body weights of the samples varied considerably among the different sampling periods and since the metabolic rate may be a size-dependent variable (Dunlap, 1969, 1971) in these frogs, regression equations were calculated following a log-log

transformation of the data and were compared. The initial hypothesis being tested is that one regression line can be used for all seven sets of seasonal data (Ostle, 1963). If the hypothesis is rejected, the hypothesis of equality of the slopes (regression coefficients) of the lines is tested. If the latter hypothesis is accepted, the adjusted means of the sample can be compared using an analysis of covariance. The results of these tests are shown in Table II. As may be seen, only in the series of Texas samples acclimated at 5° C can all seven sets of data be represented by one regression line ($P > 0.25$). Hence, this series of samples shows no statistically significant seasonally related changes in metabolic rate. In all the other sets, the hypothesis of one regression line being adequate for all is rejected

TABLE II

Comparison of the regression lines of routine metabolic rate for samples of Acris crepitans *acclimated at 5, 15 and 25° C and determined at 15° C at different times of the year. For the Texas frogs, the samples being compared for each acclimation temperature were determined on 8 April, 29 April, 31 May, 20 July, 4 Oct., 1 Nov. and 3 Dec. For the South Dakota frogs the dates are 16 April, 7 May, 27 May, 24 July, 2 Oct., 18 Oct. and 6 Nov.*

Locality	Hypothesis being tested	Acclimation temperature								
		5° C			15° C			25° C		
		F-ratio	d.f.	P	F-ratio	d.f.	P	F-ratio	d.f.	P
Texas	One regression line?	1.268	12, 24	>0.25	3.336	12, 32	<0.005	3.314	12, 33	<0.005
	Slopes equal?	—	—		2.021	6, 32	>0.05	2.085	6, 33	>0.05
	Adjusted means equal?	—	—		4.026	6, 38	<0.005	3.899	6, 39	<0.005
South Dakota	One regression line?	4.546	12, 28	<0.001	3.433	12, 27	<0.005	3.938	10, 26	<0.005
	Slopes equal?	1.827	6, 28	>0.10	1.928	6, 27	>0.10	0.494	5, 26	>0.50
	Adjusted means equal?	6.331	6, 34	<0.001	4.200	6, 33	<0.005	8.050	5, 31	<0.0005

($P < 0.005$). Furthermore, in none of these are the slopes significantly different ($P > 0.05$) and, as might be expected, in none of these are the size adjusted means all equal ($P < 0.005$).

The regression lines within each series were compared pair by pair to determine which samples were significantly· different from each other. The results may be summarized as follows.

In the series of frogs from Texas which had been acclimated at 15° C, the mean metabolic rate of the sample of 20 July was significantly different from ($P < 0.05$) and lower than those of all other samples except the samples of 29 April and 4 October. For the Texas series which had been acclimated at 25° C, the mean metabolic rate of the sample of 8 April was significantly different from ($P < 0.05$) and higher than those of all other samples with the exception of the samples of 31 May and 3 December.

In the South Dakota series of frogs which had been acclimated at 5° C, the mean metabolic rates of the samples of 2 and 18 October were significantly different from ($P < 0.05$) and higher than those of all the others with the exception of the sample of 27 May. For the frogs acclimated at 15° C, the mean rates of the 2 October and the 27 May samples were significantly different from ($P < 0.05$) and higher than those of samples determined on 18 October and 27 July, but were not significantly different from the other samples. For frogs acclimated at 25° C, the mean metabolic rates of the samples of 16 April and 7 May were significantly different from ($P < 0.05$) and higher than than those of any of the other samples except that of 2 October. The mean rate of the latter sample, in turn, was significantly different from ($P < 0.05$) and higher than those of the samples of 18 October and 6 November but did not differ significantly from the samples of 27 May and 24 July ($P > 0.05$).

Locality effects

Comparisons of metabolism-body weight regression equations calculated following a log-log transformation of the data were made for Texas and South Dakota samples of *Acris* acclimated and determined at approximately the same time of year. Samples were compared for the following six dates; the first date in each case represents the Texas sample: 8, 16 April; 29 April, 7 May; 31, 27 May; 20, 24 July; 4, 2 October; 1, 6 November. Since the metabolic rates of frogs acclimated at 5 and 15° C and determined at any one time were not significantly different from each other for either the Texas or South Dakota animals, the regression lines of the four sets of samples (5 and 15° C acclimated frogs from both Texas and South Dakota) from each of the dates were compared. In none of the comparisons could the hypothesis that one regression line can be used for all four samples be rejected ($P > 0.05$). In only two of the sets was $P < 0.10$. These were the October comparisons in which the South Dakota samples tend to have a higher metabolic rate (120 $\mu l/g$ per hr) than the Texas samples (100 $\mu l/g$ per hr) and the November samples in which the metabolic rates of frogs from both localities average somewhat lower when acclimated at 5° C than when acclimated at 15° C. No significant differences between the regression lines were found for the 25° C acclimated frogs in the 8, 16 April ($P > 0.10$); 31, 27 May ($P > 0.25$) or 1, 6 November ($P > 0.25$) samples. The warm acclimated Texas and South Dakota samples were significantly different for the 29 April, 7 May ($P < 0.0005$), July ($P < 0.025$) and October ($P < 0.05$) comparisons with the mean metabolic rates of the South Dakota samples being the higher.

Metabolic rates of Texas frogs determined at 25° C

Early April, late April and early December samples of Texas frogs which had been acclimated at 5, 15 and 25° C were determined at 25° C as well as at 15° C and the means of the different acclimation groups on each determination date were compared using Student's t-test. The mean metabolic rate for each treatment of samples determined at both 15 and 25° C is shown in Figure 3. The purpose of the determinations at 25° C was to provide additional information on possible seasonally related changes in acclimation pattern and Q_{10} values

of the frogs. As was shown earlier (Table I), statistically significant acclimation effects are apparent in April frogs when the metabolic rates are determined at 15° C. At that temperature frogs acclimated at both 5 and 15° C are significantly different from frogs acclimated at 25° C but not from each other. However, when both sets of April samples are determined at 25° C, only the frogs acclimated at 5° C differ significantly from the 25° C frogs ($P < 0.02$). The metabolic rates of frogs acclimated at 15° C do not differ significantly from either the 5 or the 25° C samples ($P > 0.10$). As was true for the frogs determined at 15° C, no significant acclimation effects can be demonstrated for the December samples determined at
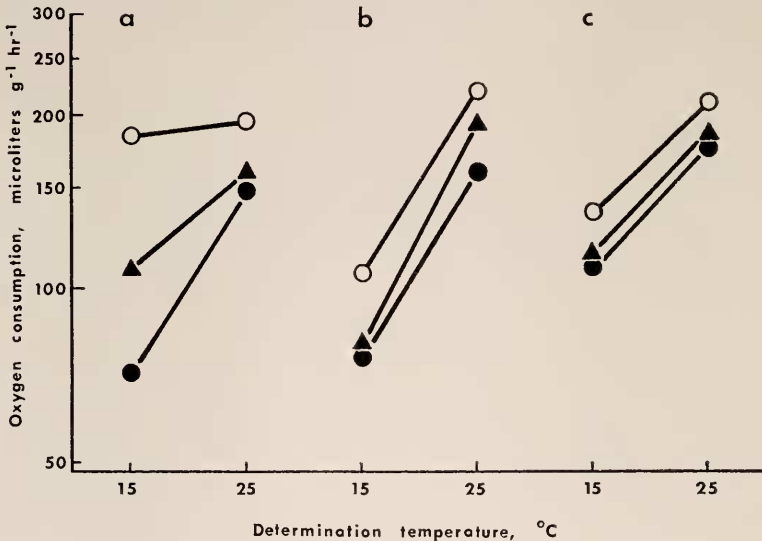

FIGURE 3. Routine oxygen consumption in *Acris crepitans* from Texas on three different sampling dates determined at 15 and 25° C following acclimation at 5, 15 and 25° C. Determinations were made on April 8–9 (a); April 29–30 (b); December 3–7 (c). Each point on the graph represents the mean metabolic rate of six frogs at the indicated determination temperature. Symbols are as in Figure 1.

25° C ($P > 0.10$) although the metabolic rates of the frogs acclimated at 25° C average higher than those acclimated at 5 or 15° C. Thus the pronounced inverse compensation shown at lower determination temperature is replaced by either a weak or no acclimation effect at 25° C.

Q_{10} values for the acutely measured rate-temperature (R-T) curves between 15 and 25° C shown in Figure 3 fall into three groups: low (1.1) for the 25° C frogs on 8–9 April; intermediate (1.5–1.6) for all three acclimation groups in December and the 15° C frogs in early April; and high (2.1–2.4) for all acclimation groups on 29–30 April and the 5° C frogs on 8–9 April. The 29–30 April values agree with July samples (Dunlap, 1972) in having Q_{10} values of greater than 2.0 between 15 and 25° C. Thus the Q_{10} values between 15 and 25° C for the acutely measured R-T curves tend to be relatively uniform and high for the late April and July frogs, uniform and intermediate for the December frogs and

variable for the early April frogs. In the latter series, the Q_{10} values are inversely proportional to the acclimation temperature with the Q_{10} value of the warm acclimated frogs approximating 1.0. The Q_{10} values for the acclimated R-T curves between 15 and 25° C tend to be higher than those of the corresponding acute curves, averaging 2.8 in late April and July and 1.8 in early April and December.

DISCUSSION

Although the seasonal metabolic cycles of the two populations differ in detail, they do share a number of features in common. On any one date at each locality, no statistically significant differences could be demonstrated between the frogs acclimated at 5 and those acclimated at 15° C when both were determined at 15° C. Furthermore, the metabolic rates of frogs from Texas and South Dakota which had been acclimated at 5 and 15° C were statistically indistinguishable when measured at about the same time of year. The timing of the two small metabolic peaks on the seasonal curves for the 5 and 15° C frogs (Fig. 1 and 2) in late May and early October is similar for the two populations. For both populations, the metabolic rates of the warm acclimated spring and summer animals are higher than those of the frogs acclimated at 5 and 15° C when all are determined at 15° C. Consequently, the pattern of inverse compensation at 15° C reported for *Acris* in earlier papers (Dunlap, 1969, 1971 and 1972) persists throughout much of the spring and summer at both localities. Both populations also exhibit a pronounced early (8 April, Texas or 16 April, 7 May, South Dakota) spring peak in the metabolic rates of warm acclimated frogs.

Although there are no clearcut differences in the metabolic rates of the two populations for frogs acclimated at 5 and 15° C, the rates of warm acclimated frogs average higher for South Dakota frogs than for Texas animals. From late May to October for the South Dakota frogs and from late April to November for the Texas frogs, the metabolic rates of the warm acclimated frogs are essentially uniform averaging about 160 μl O_2/g per hr for the South Dakota frogs and about 110 μl O_2/g per hr for the Texas frogs. Earlier in the spring, however, rates are higher, averaging about 210 μl O_2/g per hr for the South Dakota frogs and 180 μl O_2/g per hr for the Texas animals. In the autumn, the metabolic rates of the Texas frogs remain constant or perhaps increase slightly. In the South Dakota frogs, on the other hand, the metabolic rates of all the samples decrease markedly after early October. The decrease is especially pronounced for the warm-acclimated animals and by late October and early November the metabolic rates reach the lowest levels found in this study. Although data are not available for the winter months, the pattern suggests that the metabolic rates of the warm acclimated Texas frogs may increase during the winter, reaching a peak in or prior to early April. In the South Dakota animals, the metabolic rates of all three acclimation groups may continue to drop through November. Dunlap (1969) reported very low rates of metabolic activity in both warm and cold acclimated South Dakota *Acris* in early April. This suggests that in winter the frogs maintain low metabolic rates even at moderate temperatures, then, after emerging from hibernation "switch" to a higher metabolic level as found by 16 April in this study.

Precht (1958) points out that the many documented cases of seasonal and latitudinal variation in metabolic rates as well as short-term acclimation in these rates are apparently adaptive. He suggests that their biological significance lies in their enabling the same animal at different times, or populations in different localities, to at least partially stabilize the rates of vital functions largely independent of environmental temperature. In view of the evidence presented by Precht and many others, it seems reasonable to expect that the clearcut seasonally related changes in the rates of oxygen consumption in *Acris* and the consistent differences between the populations of *Acris* are adaptive. Consequently, we shall examine the available information with respect to seasonal temperature trends and the relations between body and ambient temperature in natural populations of *Acris* to see whether they show any consistent relationship to the metabolic data presented in this paper.

One of the striking features of the metabolic rate-time of year curves shown in Figures 1 and 2 is the consistently higher metabolic rates of frogs acclimated at 25° C as compared to those acclimated at the lower temperatures throughout the spring and summer. In the fall of the year the pattern changes with acclimation effects becoming much less pronounced. Consequently, before we can understand what significance these metabolic rate-temperature relationships have for field populations, we need to know when the frogs are most likely to be warm acclimated and when they are likely to be cold acclimated. To gain an insight into this problem we need information (1) on the normal local variations in the temperatures to which the frogs are subjected, (2) on the thermo-regulatory abilities of *Acris* under varying temperature regimes, and (3) on the temperatures to which the frogs acclimate when subjected to daily thermoperiods in body temperature.

Information on the first point is provided in Figures 1 and 2. These figures show, in addition to the metabolic rates, the mean daily maximum and minimum temperatures for Vermillion, South Dakota (Spuhler, Lytle and Moe, 1967) and Austin, Texas (Blood, 1960) for each month for which metabolic data are available.

Fitch (1956) and Brattstrom (1963) have presented data which are pertinent to the second point. They show that the body temperature of *Acris* fluctuates less, both seasonally and daily, than might be expected on the basis of environmental temperatures alone. Fitch reported that, for Kansas *Acris*, over half of the body temperatures of 102 frogs recorded throughout the year were between 28.0 and 31.7° C. On cool sunny days he found that a frog's temperature may exceed air temperature by 10° C or more. In warm weather, they basked less and were usually nearer air temperature. Brattstrom, using his own and Fitch's data, is in general agreement with Fitch and adds information concerning nocturnal thermo-regulation. In summary, their data suggest that on sunny days in spring and autumn when maximum air temperatures reach or exceed 15° C, basking temperatures of 25° C or more may be attained. Throughout much of the spring and summer basking temperatures of 25–30° C are common and at night, body temperatures drop to levels that, on the average, lie between ambient air temperature and water temperature.

Information relating to the third point, *i.e.,* the temperature to which the frogs acclimate when subjected to a variable thermal environment, is provided by several investigators. Heath (1963) working on the fish, *Salmo clarki,* and Hutchison and Ferrance (1970) and Seibel (1970) working on the frog, *Rana pipens,* reported that when the animals were subjected to a daily thermo-period, the critical thermal maximum acclimated to the maximum temperature during the period. Consequently, it seems reasonable to expect that the same relationship may apply with respect to acclimation in metabolic rates in *Acris.*

Based on the above information we will assume that *Acris* acclimates to the basking body temperature rather than the lower body temperatures found at night and that in sunny weather whenever air temperatures reach or exceed 15° C, basking temperatures of 25° C or more may be attained. Consequently, as may be seen from the curves for the mean maximum daily temperatures (Figs. 1 and 2), Texas frogs could, on the average, be expected to be warm acclimated throughout the period from early April to October and also during periods of clear, sunny weather later in the season. The South Dakota frogs would usually be warm acclimated from early June to September and, in clear weather, from April into October. If this admittedly simplified analysis is at all accurate then the curves of the warm acclimated frogs are ecologically the most meaningful of the curves throughout much of the study period.

Dunlap (1972) has presented evidence for a zone of metabolic insensitivity to temperature on the acutely measured metabolic rate-temperature (R-T) curves for warm-acclimated *Acris* from Texas and South Dakota. This zone extends between body temperatures of 15–25° C for South Dakota frogs and between 20–30° C for Texas frogs in July. The mean minimum daily temperature for July for each locality lies within the range of temperatures for which metabolic stability can be maintained. Conseqeuntly, it was suggested that this R-T pattern, together with the thermoregulatory ability of *Acris,* reflects an adaptation enabling the frogs to maintain high and constant metabolic rates even in the face of rather wide ranges in environmental temperature between night and day and from day to day. It was further suggested that each population was adapted to the range of temperature, particularly the normal minimal temperature the frogs are exposed to during the daily temperature cycle. This was suggested not only by the shift of the metabolic plateau in South Dakota frogs some 5° C to the left relative to that of the Texas frogs, but also by the fact that the entire warm acclimated R-T curve extending between 5 and 35° C was so shifted. Consequently, Texas frogs which were acclimated and determined at 15° C had uptakes that were lower than those of South Dakota frogs determined at 15° C but similar to those of warm acclimated South Dakota frogs determined at 10° C. Similarly, South Dakota frogs determined at 15° C had uptakes that were similar to those of Texas frogs determined at 20° C.

The data presented in the present paper show that, for warm acclimated frogs, the oxygen uptake at 15° C averages about 50 μl/g per hr greater for the South Dakota frogs than for the Texas frogs from late May to early October. Throughout the bulk of this period, mean minimal daily temperatures lie near or above 20° C on a monthly basis for Austin and near or above 15° C for Vermillion. As seen earlier, the South Dakota frogs at 15° C have metabolic rates similar to those

of Texas frogs at 20° C. This, together with the insensitivity of the metabolic rates to temperature over a wide temperature range above 15° C (South Dakota) or above 20° C (Texas), would result in the metabolic rates of the two populations being about the same throughout the late spring and summer in spite of the lower average temperatures to which the South Dakota populations are exposed.

Marked seasonal changes in the metabolic rates of intact frogs of several species of *Rana* (Krogh, 1904; Dolk and Postma, 1927; Stangenberg, 1955; and Fromm and Johnson, 1955) and in *Acris crepitans* (Dunlap, 1969) have been reported. The seasonal changes frequently include a period in the spring when metabolic rates are considerably higher than at other seasons. This period of high oxygen consumption has usually been considered to be related to an increase in activity associated with the spawning season. However, reproductive activity alone is insufficient to account for the early spring peaks in metabolic rates in *Acris*. The peak in breeding activities in both populations occurs after the early spring metabolic peak when metabolic rates have dropped to the summer levels. In South Dakota for example, the metabolic rates are high in late April and early May but have dropped to the summer levels by the end of May. In this area, male *Acris* frequently begin calling by the middle of May, but the major breeding choruses are found in later May and in June (Dunlap, personal observations). In the Texas frogs, high metabolic rates were found in early April but by the end of April the rates had dropped to summer levels. Near Austin, Texas, although chorusing may begin in late January or February, the first records of amplexus were not found until the middle of March and breeding may continue at intervals throughout the summer (Blair, 1961).

An alternative explanation for the early spring metabolic peak in *Acris crepitans* is that it simply represents another example of metabolic compensation for temperature. This results in the maintenance of the metabolic rates of early spring animals at summer levels even though environmental temperatures, and presumably body temperatures, are below summer levels.

As was pointed out earlier (Dunlap, 1972), metabolic rates for warm acclimated frogs at temperatures of 10° C for the South Dakota and 15° C for the Texas frogs are approximately the same for the two populations and average about 50 μl O_2/g per hr less than the stabilized summer rates. These temperatures are also about 5° C below the lower threshold temperatures permitting the stabilized summer rates. If the mean minimal body temperature for active *Acris* in late April and early May in South Dakota and in March and early April in Texas averaged about 10 and 15° C, respectively, then the high rate of oxygen consumption at that time would compensate for the lower temperatures. This is most readily seen from the Texas seasonal curve (Fig. 2). On that curve, the mean rate on 8 April determined at 15° C (180 μl O_2/g per hr) is almost identical to the July rates between 20–30° C (Dunlap, 1972). Although the mean minimal air temperature in April is a little less than 15° C, Brattstrom (1963) found that at night when water temperatures were higher than air temperatures *Acris* would thermoregulate by moving alternately between the water and the shore and consequently body temperatures averaged somewhat above air temperature. The rates of South Dakota frogs determined at 15° C on 16 April and 7 May are even higher than the July rates between 15 and 25° C (Dunlap, 1972). This sug-

gests that the compensation is for a greater than 5° C temperature differential and indeed, the mean minimal temperature for Vermillion in April is well below 10° C. Much more information is needed on the ranges of body temperatures that are characteristic of the frogs in early spring before a more accurate analysis can be made.

A possible model for this switch in the metabolic response to temperature exists in the same apparent mechanism described for the difference in metabolic responses between northern and southern populations (Dunlap, 1972), *i.e.,* a translation of the acute R-T curves to the left in the early spring and back toward the right in summer frogs. Such a change might be mediated by response to photoperiod or perhaps, to a photoperiod-temperature interaction. This interpretation is supported for the Texas frogs by the data shown in Figure 3. Here it can be seen that the Q_{10} for warm acclimated frogs is approximately 1.0 between 15 and 25° C in the early April samples while it is over 2.0 in the later April and in the July samples (Dunlap, 1972). This suggests that the plateau of metabolic insensitivity between 20 and 30° C in the July frogs has been shifted at least 5° C to the left in the early spring frogs.

In October changes occur in the effects of acclimation on the metabolic rates of both Texas and South Dakota frogs. The changes, however, are different for the two populations. During this period the metabolic rates of Texas frogs which are acclimated at 25° C exhibit little change from the summer rates. However, the mean metabolic rates of those acclimated at 15° C and, to a lesser extent, those of frogs acclimated at 5°C, rise to levels approximating those of warm acclimated frogs. In the frogs from South Dakota, there is an increase in the mean metabolic rates of the frogs acclimated at 5 and 15° C in early October followed by a sharp drop in the rates of all acclimation groups to levels approximating those of cold acclimated spring frogs.

As a result of this change in pattern, the Texas frogs can maintain relatively high metabolic rates on warm days in the fall and winter regardless of the temperature to which they have previously been acclimated. This metabolic pattern would seem to be consistent with the activity patterns which have been reported for *Acris* in Texas. Thus, Pyburn (1958) reported that in the vicinity of Austin, Texas, *Acris* is active throughout the year although the number of active frogs decreases in December and January.

The increase in the metabolic rates of cold acclimated South Dakota *Acris* noted in early October may have an adaptive significance similar to that of the pattern noted in the Texas frogs. That is, it would allow the frogs to be active on warm autumn days even though they had been cold acclimated. The abrupt drop in metabolic rates regardless of the acclimation history noted in South Dakota frogs later in the season may well be related to the conservation of energy reserves during the period prior to hibernation and during hibernation. The reduction of metabolic rates in South Dakota *Acris* in the winter is consistent with the 4–5 month period of hibernation in these frogs (Dunlap, 1971).

SUMMARY

Samples of cricket frogs were collected in South Dakota and Texas at intervals throughout the spring, summer and autumn and acclimated for 5–7 days at 5, 15 and 25° C. Routine metabolic rates were determined at 15° C for acclimated frogs from both localities. Sample of Texas frogs collected in early April, late April and December were determined at 25° C as well.

There were no statistically significant differences between the means of samples acclimated at 5 and 15° C and determined at 15° C on any date for either the South Dakota or the Texas frogs. Except for Texas frogs acclimated at 25 and 5° C in November, no significant differences were found among the means of the three acclimation groups from either locality from October into December. From April through July, with the exception of the Texas samples of 31 May, the frogs from both localities which were acclimated at 25° C had metabolic rates which were consistently higher than and significantly different from frogs acclimated at either 5 or 15° C.

When frogs which had been collected at different times of the year and acclimated at the same temperature were compared, significant seasonal effects were found in all series except the Texas frogs acclimated at 5° C. Several pronounced seasonally related patterns were found. Low metabolic peaks were found in the 5 and 15° C acclimated frogs in late May and early October in both Texas and South Dakota frogs. In the South Dakota frogs acclimated at 25° C, the metabolic rates were highest in April and early May. The rates dropped somewhat in late May and remained fairly uniform until early October. In later October and November the metabolic rates of the warm acclimated frogs from South Dakota reached the lowest levels of the year. In the Texas frogs acclimated at 25° C and determined at 15° C, rates were highest in early April, dropped off by early May and remained fairly uniform into November. There was an indication of a slight increase in the metabolic rates of the warm acclimated frogs in December.

The metabolic rates of Texas and South Dakota frogs with the same acclimation history were compared at approximately the same time of year. In no case were there significant differences attributable to locality for frogs acclimated at either 5 and 15° C. For frogs acclimated at 25° C, the mean metabolic rates of the South Dakota frogs were consistently higher than those of seasonally comparable Texas frogs from April to October and showed statistically significant differences for the 29 April–7 May, July and October comparisons.

For the Texas frogs, the presence of inverse acclimation shown in the April frogs at 15° C with the warm acclimated animals having higher metabolic rates than those acclimated at 5 or 15° C, is replaced by either a weak or no acclimation effect at 25° C.

The results of this study suggest that the pattern of inverse compensation at 15° C reported for *Acris* in earlier papers persists throughout much of the spring and summer in both the South Dakota and the Texas populations. The data further demonstrate the tendency for the metabolic rates of warm acclimated

frogs from South Dakota to be higher at determination temperatures of about 15° C than comparable rates in Texas frogs. The seasonally related differences in the metabolic rates and also the differences in the metabolic rates of frogs from the two localities are interpreted in terms of published data on body temperature-environmental temperature relationships in *Acris* and with published climatological data. The data are considered to be consistent with the hypothesis of the role of metabolic patterns in the maintenance of metabolic stability in thermally instable environments.

LITERATURE CITED

BLAIR, W. F., 1961. Calling and spawning seasons in a mixed population of anurans. *Ecology*, **42** : 99–110.

BLOOD, R. D. W., 1960. Climates of the states; Texas. No. 60–41, U.S. Dept. Commerce Weather Bureau, *Climatography of the United States*. U.S. Government Printing Office, Washington, 28 pp.

BRATTSTROM, B. H., 1963. A preliminary review of the thermal requirements of amphibians. *Ecology*, **44** : 238–255.

BULLOCK, T. H., 1955. Compensation for temperature in the metabolism and activity of poikilotherms. *Biol. Rev.*, **30** : 311–342.

DOLK, H. E., AND N. POSTMA, 1927. Über die Haut und die Lungenatmung von *Rana temporaria*. *Z. Vergl. Physiol.*, **5** : 417-444.

DUNLAP, D. G., 1969. Influence of temperature and duration of acclimation, time of day, sex and body weight on metabolic rates in the hylid frog, *Acris crepitans. Comp. Biochem. Physiol.*, **31** : 555–570.

DUNLAP, D. G., 1971. Acutely measured metabolic rate-temperature curves in the cricket frog, *Acris crepitans. Comp. Biochem. Physiol.*, **38A** : 1–16.

DUNLAP, D. G., 1972. Latitudinal effects on metabolic rates in the cricket frog, *Acris crepitans:* Acutely measured rates in summer frogs. *Biol. Bull.*, 143 : 332–343.

FITCH, H. S., 1956. Temperature responses in free-living amphibians and reptiles of northeastern Kansas. *Univ. Kansas Publ. Mus. Natur. Hist.*, **8** : 417–476.

FROMM, P. O., AND R. E. JOHNSON, 1955. The respiratory metabolism of frogs as related to season. *J. Cell. Comp. Physiol.*, **45** : 343–359.

FRY, F. E. J., 1957. The aquatic respiration of fish. Pages 1–63 in M. E. Brown, Ed., *The Physiology of Fishes*, Vol. 1. Academic Press, New York.

HEATH, W. G., 1963. Thermoperiodism in sea-run cutthroat trout (*Salmo clarki clarki*). *Science*, **142** : 486–488.

HUTCHISON, V. H., AND M. R. FERRANCE, 1970. Thermal tolerances of *Rana pipiens* acclimated to daily temperature cycles. *Herpetologica*, **26** : 1–8.

HUTCHISON, V. H., W. G. WHITFORD AND M. KOHL, 1968. Relation of body size and surface area to gas exchange in anurans. *Physiol. Zool.*, **41** : 65–85.

JAMESON, D. L., W. TAYLOR AND J. MOUNTJOY, 1970. Metabolic and morphological adaptation to heterogenous environments by the Pacific tree toad, *Hyla regilla. Evolution*, **24** : 75–89.

LI, J. C. R., 1957. *Introduction to Statistical Inference.* Edwards Brothers, Ann Arbor, 568 pp.

KROGH, A., 1904. On the cutaneous and pulmonary respiration of the frog. (A contribution to the theory of the gas exchange between the blood and the atmosphere.) *Skand. Arch. Physiol.*, **15** : 328–419.

OSTLE, B., 1963. *Statistics in Research.* (2nd. Ed.) Iowa State University Press, Ames, 585 pp.

PRECHT, H., 1958. Concepts of the temperature adaptation of unchanging reaction systems of cold-blooded animals. Pages 50–77 in C. L. Prosser, Ed., *Physiological Adaptations.* American Physiological Society, Washington.

PROSSER, C. L., 1955. Physiological variation in animals. *Biol. Rev.*, **30** : 229–262.

PROSSER, C. L., 1958. General summary: The nature of physiological adaptation. Pages 167–
     180 in C. L. Prosser, Ed., *Physiological Adaptations,* American Physiological So-
     ciety, Washington.
PYBURN, W. F., 1958. Size and movements of a local population of cricket frogs (*Acris
     crepitans*). *Texas J. Sci.,* **10** : 325–342.
SEIBEL, R. V., 1970   Variables affecting the critical thermal maximum of the leopard frog,
     Rana pipiens Schreber. *Herpetologica,* **26** : 208–213.
SPUHLER, W., W. F. LYTLE AND D. MOE, 1967. *Climatological Summary No. 8; Vermillion,
     South Dakota.* Agricultural Experiment Station, South Dakota State University,
     Brookings, 6 pp.
STEBBINS, R. C., 1951. *Amphibians of Western North America.* University of California
     Press, Berkeley and Los Angeles, 539 pp.
STANGENBERG, G., 1955. Der Temperatureinfluss auf Lebensprozesse und den Cytochrom c-
     Gehalt beim Wasserfrosch. *Pfluegers Arch. Gesamte Physiol. Menchen Tiere,* **260** :
     320–332.
TASHIAN, R. E., AND C. RAY, 1957. The relation of oxygen consumption to temperature in
     some tropical, temperate and boreal anuran amphibians. *Zoologica,* **42** : 63–68.
VERNBERG, F. J., 1962. Comparative physiology: Latitudinal effects on physiological properties
     of animal populations. *Annu. Rev. Physiol.,* **24** : 517–546.